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EFFECT OF MOBILE PHONE RADIATIONS ON PROTEIN CONTENT OF SOYBEAN SEEDS

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ABSTRACT

The use of mobile phone has increased with the modernization. It has lead to the increase in the radiations in the environment which further has effect on the plants and animals including Human beings. The investigation was based on the effect of mobile phone radiations on the Protein Content of Soybean. In the current study the investigation was done that whether the mobile phone radiations has positive effect or negative effect on the Protein content and various other parameters including morphological parameters like Germination percentage, Seedling length, Fresh Weight and Dry weight. The result after experiments show that the radiations have negative effect on the morphological parameters like Germination percentage, Seedling length, Fresh Weight and Dry weight i.e. a reduction was observed in all the morphological parameters due to the oxidation stress as a result of which the reactive oxygen species were released that results in the change of membrane composition and its degradation and thus the reduction was reported. But on the other hand the positive effect was recorded in the Soybean Protein Content which was due to release of phenolic compounds (antioxidative) which results in increase in the protein content. The radiation causes the oxidative stress due to which the enzymes activity increases in the cells both Lipid peroxidase and Guaiacol peroxidase activity.

KEYWORDS: *Irradiated Seeds*, Soybean, Guaiacol Peroxidase (GPX), Oxidative Stress, Mobile Phone Radiations

INTRODUCTION

With the invention of new technologies and modernization in the way of living many devices are used which have an undesirable effects on environment including human being, plants and animals. One of these is radiations, which are emitted by the mobile phones i.e. microwaves. These are in very much use in the present day situation like in broadcasting and telecommunication. Many scientific studies have investigated possible health symptoms of mobile phone radiation (**Ruediger, 2009)**. There are some evidences that microwaves produces changes in the plants also. The cell membrane's permeability and cell growth is affected by radiations. It also shows interference with ions and organic molecules like Proteins. Radiations are known to induce physiological and genetic modifications like production of various epidermal meristems in the hypocotyl, modifications of the proteome etc. Decrease in seed germination was observed in the seed samples with increase in time of exposure of radiations. With increase in frequency the plant height and root length was observed to decrease where as biomass % was increased significantly (**Ragha *et al.*, 2011)**. In the present study, the investigation was based on the effect of radiations on the Protein content of soybean seeds on the basis of difference in frequency and difference in period of exposure of radiations to soybean seeds. Mobile phone use electromagnetic radiations in the microwave range. Microwaves are non-ionizing radiations with wavelengths ranging from as long as one meter to as short as one millimeter and the frequency ranges between 300MHZ to 300 GHZ. The two types of radiations are differentiated by the way they interact with normal chemical matter: ionizing and non-ionizing radiation.

The word radiation is often used in reference to ionizing radiation, but the term radiation may correctly also refer to non-ionizing radiation (e.g., radio waves, heat or visible light) as well.

Ionizing radiations are more harmful to living organisms than non-ionizing radiations as these produce ions even at low radiation powers and have the potential to cause DNA damage. Non-ionizing radiation is considered harmless at low power as cannot produce a significant temperature rise **(Foster *et al.*, 2004).** Non-ionizing electromagnetic radiation has low energy and the energy is only sufficient to change the rotational, vibrational or electronic valence configurations of molecules and atoms. This produces thermal effects. In such cases, even "non-ionizing radiation" is capable of causing thermal-ionization only if it deposits enough heat to raise temperatures to ionization energies. These reactions occur at far higher energies than with ionizing radiation, which requires only single particles to ionize**. (Schuz *et al.*, 2006.)**

The botanical name of Soybean is *Glycine max*. The word *Glycine* is derived from the Greek – *glykys* (sweet). Soybean is a cheap source of protein. Soy varies in growth and habit. The height of the plant varies from less than 0.2 to 2.0 m. The fruit grows in clusters of three to five and each pod is 3–8 cm long.The pods are covered with fine brown or gray hairs. The leaves are trifoliate having three to four leaflets per leaf, and the leaflets are 6–15 cm long and 2–7 cm broad. **(Endres, 2012).** Soybeans are an important global crop, providing oil and Protein. Cultivation is successful in climates with hot summers, when temperatures range from 20 to 30 °C, temperatures below 20 °C and over 40 °C may retard growth significantly. Soybean contains about 20 per cent oil and 40 per cent high quality Protein. Soybean protein is rich in amino acid lysine in which most of the cereals are deficient.

In also contains a good amount of minerals, salts and vitamins like thiamine and riboflavin and its sprouting grains contain a considerable amount of Vitamin C (**Liener *et al., 1992***). The amount of both protein and fat is higher in Soybeans but, is relatively low in carbohydrates. Soy protein is also of the highest quality. Soybean Protein consist of all essential amino acids and thus is a complete protein. The protein present in Soybean is Glycinin and beta-conglycinin.**(Abd. EM *et al*., 2011).** The Soybean is the important legume or pulse as it contains a higher amount of protein in it that is equal to the animal meat protein. And thus it forms a highly nutritional food for the vegetarian especially for its high protein content. So the experiment was performed to check whether the mobile phone radiations cause harmful or useful effects on the Soybean seeds and especially on its protein content.

The experiment investigates the *in vitro* effect of 2G and 3G mobile phone radiations on Morphological parameters of Soybean i.e. Germination %, seedling length, fresh weight and dry weight and check the effect of 2G and 3G mobile phone radiations on Biochemical parameters of Soybean seedling i.e. Lipid Peroxidation test, Protein estimation test, Guaiacol peroxidation test.

MATERIALS AND METHODS

Chemicals and Instruments

Tricarboxylic acid (TCA), Thiobarbituric acid (TBA),Guaiacol peroxidase were purchased from Himedia, Mumbai. Folin Ciocaeteu's phenol reagents were purchased from SRL Pvt.Ltd., Mumbai, India. The instruments Centrifuge (REMI Instrument Ltd. Mumbai, India), Hot air Oven (Microsoft India), Autoclave (NSW Pvt. Ltd. New Delhi, India), Spectrophotometer (Systronics, Ahmedabad) were used in the experiments.

Seeds Collection

The seeds were obtained from the **Punjab Agricultural University, Ludhiana, India**.The seeds of variety ***Glycine max var. SL525*** were obtained for the experiment. Seeds of Soybean were taken and imbibed in distilled water for 8 h, placed in air tight plastic boxes lined with filter paper moistened with D/W. A Nokia 2690 mobile phone with frequency band 850- 1850 MHz was used to irradiate the seeds and one seeds sample was taken as control to compare the effect of radiation on Soybean seeds. Different time of exposure was given to seeds so as to see the effect of radiations like ½ hour, 1 hour, 2 hours, 4 hours and 8 hours. After this the seeds were left for germination at least for 72 hours and then further tests were conducted to check the effect of radiation on Soybean. For difference in frequency the other set of soybean seeds was irradiated by the phone having 3G technologies. In this set, seeds were irradiated through Samsung GT B7722 with frequency band 900- 1900MHz. In this set up also one sample was taken as control in which no radiations were given and in other sample radiations were given with difference in time period. Similarly the procedure of morphological and biochemical analysis was performed.

Morphological Analysis

After 72 hour of radiations, germination percentage of seeds was recorded as the number of seeds germinated, seedling length was estimated through length of plumule and fresh weight was recorded by weighing all the seeds. Same seedlings of fresh weight were allowed to dry at 70^0C for 24 h to record the dry weight.

Biochemical Analysis

Estimation of Lipid Peroxidation

For Lipid Peroxidation test, the 0.2 g of seedlings were homogenized by addition of 1ml of 5% TCA solution using pestle and mortar. Homogenate was centrifuged at 12000 rpm for 15min. at room temperature. To the 1 ml of supernatant 4 ml of 0.5% Thiobarbituric Acid in 20% TCA solution was added and after that the sample was incubated at 96^0C for 30 min. immediately the test tubes were kept in ice bath and then centrifuged at 2000 rpm for 10 min. The absorbance was recorded at 600 nm.

Extraction of Protein

Leaves (2g) were taken and homogenized in 4 ml of 0.05 M Phosphate buffer (1.7g monobasic sodium phosphate + 1.1g of Dibasic Sodium phosphate in 250ml water). After this the centrifugation was done at 12000 rpm for 30 minutes at 4°C. Then supernatant was collected for estimation.

The 0.5ml of supernatant was transferred to a glass tube and 0.7ml Lowry solution was added in the tube. After this the tubes were covered and incubated for 20 min. In the last five min. Folin reagent was prepared fresh. After 20 min of incubation the samples were taken out and addition of 0.1ml of diluted Folin reagent was done. Incubation was done once again for 30 min at room temp. After 30 min the sample was transferred into cuvette and optical density was taken at 750nm. Absorbance of this mixture was recorded against the Bovine Serum Albumin (BSA). Standard curve was prepared by using BSA (10-100 micro gram /ml).

Estimation of Guaiacol Peroxidase (GPX)

GPX activity was determined in term of increase in absorbance at 470 nm due to oxidation of guaiacol. The seeds sample was crushed and 50 µL of sample was taken in a test tube. To the sample addition of sodium phosphate buffer,

Hydrogen peroxide and Guaiacol was done. Then the reaction mixture was incubated for 8 minutes. The absorbance was recorded at 470nm **(Afzal and Mansoor, 2012).**

RESULTS

Morphological Analysis

In the morphological analysis reduction in the germination percentage, seedling length was observed with an increase in the fresh weight and dry weight of the seeds sample for the 2G mobile phone, whereas in case of 3G mobile phone exposed samples decrease in all the parameters was recorded like germination percentage, seedling length, fresh weight, dry weight. Three replicates for each parameter were taken and designated as $R_{1,}$ $R_{2,}$ and R3.. The mean of readings was taken and standard deviation was calculated for finding the significance of results obtained.

Biochemical Analysis

The readings of Biochemical tests were checked out for their significance through Graph **Pad Prism version 5,** the analysis method used was **One Way Anova.** The readings were significant as they have value $P \leq 0.05$.

Lipid Peroxidation Test

In the 2G tests initially there was an increase in the lipid peroxidation activity of irradiated seeds but with increase in the time period the peroxidation activity was decreased in comparison to controlled seeds. In the 3G tests there was an increase in the lipid peroxidation activity of irradiated seeds in comparison to controlled seeds sample.

Protein Estimation Test

In the protein estimation tests, in case of radiations from 2G mobile phone initially there was an increase in the O.D. and after 1 hour radiation there was seen a decrease, but in comparison to control the O.D. of radiated sample has increased. In case of radiations from 3G mobile phone the increase in the O.D. was recorded. After taking the O.D. the Protein content was measured.

Guaiacol Peroxidation

In the Guaiacol peroxidation tests, an increase in the activity of guaiacol peroxidase was seen after the 2G and 3G tests.

DISCUSSIONS

Various studies have documented the positive and the negative effects of radiations on plants and animals and it was found out that the radiations cause different effects on human being and also on the plants. The radiations cause its effect by the dielectric heating effect which causes the temperature to raise which results in electrical conductivity of most biological tissues.

In the present study we found that the mobile phone radiations cause a change in plant morphology and biochemistry. In the past experiment was performed by **Afzal and Mansoor** in 2012 on the plants which show the reduction in germination percentage, seedling length, weight i.e. fresh and dry weight of irradiated seeds and similar results were reported in present study. They reported that the mobile phone radiations significantly reduced the seedling length and dry weight of seeds after exposure for 0.5,1, 2, and 4 h.The reduction in seed germination, root length and biomass % was seen for most of the samples when used with increase in microwave power and exposure time as compared to control

by **Ragha 2011.** Irradiation provoked insignificant changes in lipid peroxidation and soluble protein content, while protein oxidation intensity was significantly decreased when dose of 10 kGy was applied. Presented results implicated that increased antioxidant capacity and protein stability of soybean seeds were increased after application of irradiation (**Moneim, 2011).**

In present study it was found that the increase in radiation causes a decrease in various parameters like germination percentage, seedling length, fresh weight and dry weight. An increase in the enzyme activity like of lipid peroxidase activity increases in case of radiations from 3G mobile phone from range 0.105-0.139 and that of Guaiacol peroxidase ranges from 0.764-0.781 in case of radiations from 2G mobile phone and in case of radiations from 3G mobile phone it ranges from 2.222-2.470. The Protein content of seeds was recorded to increase from range 0.166- 0.231 in case of radiation from 3G mobile phone. From the present study it can be concluded that the mobile phone radiations cause a decrease in the morphological parameters like germination percentage, seedling length, fresh weight and dry weight of the irradiated seeds due to the oxidation stress as a result of which the reactive oxygen species were released that results in the change of membrane composition and its degradation and thus the reduction was reported in the morphological parameters of Soybean seeds. The enzyme activity increases to combat the effect of mobile phone radiations on seeds. The Protein Content of irradiated seeds was also increased on increasing time of exposure. The increase in Protein content was due to production of DPPH i.e. **-** 2,2-diphenyl-1-picrylhydrazyl. These radical scavengers act as protective agents against the protein oxidation. Some phenolic compounds are also released by cells which act as antioxidants by retarding protein oxidation reaction or by binding to the proteins. The antiradical mechanism in phenol-protein aggregates may be due to ability of phenolic compounds to transfer oxidative damage from one phenolic site to other, protecting proteins from oxidation. So, it can be concluded that the mobile phone radiations cause an increase in the activity of various enzymes like peroxidases and also cause an increase in the protein content of irradiated seeds.

CONCLUSIONS

From the present study it can be concluded that the mobile phone radiations cause a decrease in the morphological parameters like germination percentage, seed length, fresh weight and dry weight of the irradiated seeds due to the oxidation stress as a result of which the reactive oxygen species were released that results in the change of membrane composition and its degradation and thus the reduction was reported in the morphological parameters of Soybean seeds. The enzyme activity increases to combat the effect of mobile phone radiations on seeds. The activity of enzymes Lipid peroxidase and Guaiacol peroxidase was reported to increase on exposure of radiations. The Protein Content of irradiated seeds was also increased on increasing time of exposure. This reduction in the oxidation of protein content was due to production of DPHH radical scavengers which act as protective agents against the protein oxidation. So it can be concluded that the mobile phone radiations cause an increase in the activity of various enzymes like peroxidases and also cause an increase in the protein content of irradiated seeds. The results indicate that all these effects should be further evaluated and investigated for plant growth.

DECLARATION OF INTEREST

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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APPENDICES

Figure 1: Controlled Seeds Sample

Figure 2: Radiated Seeds Sample with 3G Mobile Phone

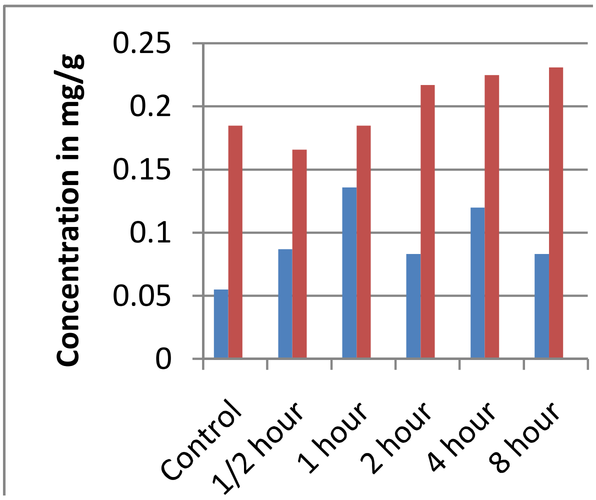

Figure 3: Protein Content in the Sample in Concentration mg/g V/S Time of Exposure of Radiations from ½ Hour to 8 Hour

Table 1: Standard Deviation Analysis for Morphological Parameters for Samples Exposed through 2G Mobile Phone Radiations

Time of Exposure	Germination (%)	Seedling Length (cm)	Fresh Weight(g)	Dry Weight (g)
Control	93.33± 11.548*	5±1*	3.25 ±1.43	0.863± 0.28
½ hour	93.33± 11.54*	5 ±1*	3.87±1.84	0.823±0.30
1 hour	86.66± 11.54	5.66± 0.57	3.62±1.67	0.926±0.28
2 hour	73.33± 5.77	4.33±0.57	3.83±1.66	1.046±0.33
4 hour	60 ±17.32	4 ±1*	3.97±1.15	1.169±0.33
8 hour	50 ± 0*	4 ±1*	3.38±0.65	1.130±0.36

Values are means of three replicates, *Non significant values

Table 2: Standard Deviation Analysis for Morphological Parameters for Samples Exposed through 3G Mobile Phone Radiations

Time of Exposure	Germination %	Seed Length (cm)	Fresh Weight (g)	Dry Weight
Control	100*	5.6±0.57	3.40±0.04*	0.98±0.017
½ hour	100*	5.3±0.57	3.31±0.09*	0.93±0.20*
1 hour	93.3±1.57	5 ±0	3.23±0.07	0.90±0.30*
2 hour	80±0	4.33±0.57	3.08±0.07*	0.88±0.02
4 hour	76.66±5.77	4±0	2.99±0.02*	0.85±0.36*
8 hour	73.33±5.77	3.66±0.57	2.92±0.02	0.82±0.03*

Values are means of three replicates, *Non significant values

Table 3: Mean Values of Sample Irradiated with 2G and 3G Mobile Phone Radiations for Lipid Peroxidation Test

Radiations	Control	½ Hour	1 Hour	2 Hour	4 Hour	8 Hour
2G	0.041^a	0.086^b	0.041^c	0.032^d	0.028^e	0.024^f
3G	0.072^a	0.105^b	0.108^c	0.123^d	0.132^e	0.139^f

Different alphabets in a column represent significant difference at p<0.05 applying Tukey’s test, Values are means of three replicates

Table 4: Mean Values of Sample Irradiated with 2G and 3G Mobile Phone Radiations for Protein Estimation Test

Radiations	Control	½ Hour	1 Hour	2 Hour	4 Hour	8 Hour
2G	0.290^{a}	1.010^{b}	1.503^{c}	0.973^{d}	1.341^{e}	0.970^{f}
3G	1.057^{a}	1.799^{b}	1.998^{c}	2.309^{d}	2.396^{e}	2.454^{f}

Different alphabets in a column represent significant difference at $p<0.05$ applying Tukey's test, Values are means of three replicates

Table 5: Mean Values of Sample Irradiated with 2G and 3G Mobile Phone Radiations for Guaiacol Peroxidation Test

Radiations	Control	½ Hour	1 Hour	2 Hour	4 Hour	8 Hour
2G	0.759^{a}	0.764^{b}	0.768^{c}	0.769^{d}	0.776^{e}	0.781^{f}
3G	1.057^{a}	2.222^{b}	2.290^{c}	2.317^{d}	2.402^{e}	2.470^{f}

Different alphabets in a column represent significant difference at $p<0.05$ applying Tukey's test, Values are means of three replicates